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Behaviour of five isopod species in standardized tests for pH preference

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Abstract

The acidity of soils is influenced by deposition from air pollution, which may cause the soil invertebrate community to change its species composition. This paper investigates the pH-preference of five species of isopods, *Oniscus asellus, Trichoniscus pusillus, Porcellio scaber, Philoscia muscorum* and *Armadillidium vulgare*, in order to identify possible bioindicators for soil acidification. The isopods were subjected to standardized testing procedures, using ring-shaped preference units with the bottom subdivided in compartments filled with sand, adjusted to pH in the range 2 to 9 by means of McIlvaine buffers. The buffer solutions were diluted to equal osmolarity for all pHs. After a series of tests, frequency distributions were obtained showing that all species have a broad distribution, with maxima at pH 5 to pH 7. The species *O. asellus, T. pusillus* and *P. scaber* are rather indifferent to acid substrates, while *A. vulgare* has the most alkaline preference and would thus seem to be useful to indicate biological effects of acidification. The sequence of acidophility of the species is grossly in line with field observations.

Keywords: pH, acidity, soil, isopods, preference, *Oniscus asellus, Trichoniscus pusillus, Porcellio scaber, Philoscia muscorum, Armadillidium vulgare.*

Comportement de cinq espèces d'Isopodes au cours de tests standardisés de préférence vis-à-vis du facteur pH.

Résumé

L'acidité du sol a été influencée par des dépôts provenant de la pollution aérienne, qui peuvent provoquer des changements dans la composition en espèces de la communauté des invertébrés du sol. Ce travail met en évidence la préférence pour le facteur pH chez cinq espèces d'isopodes, *Oniscus asellus, Trichoniscus pusillus, Porcellio scaber, Philoscia muscorum* et *Armadillidium vulgare*, dans le but d'identifier des bio-indicateurs possibles de l'acidification du sol. Les isopodes ont été soumis aux tests selon une procédure standardisée. On a réalisé les tests dans des chambres en anneau divisé à la base en compartiments remplis de sable et ajustés aux valeurs de pH dans la zone 2 à 9 au moyen de tampons de McIlvaine. Les solutions des tampons ont été diluées jusqu'à une osmolarité identique pour tous les pH. Après une série de tests, on a obtenu des distributions de fréquence, qui montrent une distribution large pour toutes les espèces, avec des maxima situés de pH 5 à pH 7. Les espèces *O. asellus, T. pusillus* et *P. scaber* sont assez indifférentes aux substrats acides, alors que *A. vulgare* montre une préférence plus alcaline; pour cette raison cette espèce paraît propre à l'indication biologique des effets de l'acidification. L'ordre d'acidophilie des espèces s'accorde en majeure partie avec les observations sur le terrain.

Mots-clés : pH, acidité, sol, isopodes, préférence, *Oniscus asellus, Trichoniscus pusillus, Porcellio scaber, Philoscia muscorum, Armadillidium vulgare.*

INTRODUCTION

The distribution of soil invertebrates is limited by various soil factors such as soil moisture, litter type, temperature, etc. Among these factors, soil pH is of particular interest, since it is presently changing due to human activities. Acidification of soils is caused by acidity of rain, volcanoes, decomposition processes in soil, excretory products from animals, etc. Soil pH will influence the distribution of soil invertebrates, but the information on this topic is mainly derived from field observations. In this paper we apply laboratory experiments in order to determine the influence of soil pH on the behaviour of isopods.

In soils affected by air pollution, the production of protons from external sources is often much greater than the natural rate of proton production in the soil; however, soils are buffered against acidification in various ways (Binckley and Richter, 1987). A decrease of pH will occur only after the acid neutralizing capacity has been seriously depleted. Nevertheless, long-term studies have shown that acid deposition over several decades may indeed change soil acidity (Tamm and Hallbäcken, 1988).

The effect of acidification may cause the pH of the soil to decrease in such a way that soil animals react to it. This may have consequences for the species composition of the soil animal community (Van Straalen *et al.*, 1988). Isopods, a group of soil animals that live on the soil surface and take part in decomposition processes may be expected to react to a change of the soil pH, although this has not been documented up to now. Other animals however, have been shown to react. For example, Hågvar and Amundsen (1981) found that the population densities of several mite species were affected by a change in soil pH induced by artificial acidification; also, Collembola populations were affected (Hågvar, 1984).

In this paper the aim will be to document the responses of several species of isopods to different pHs in the substrate. We used laboratory based standardized preference tests to identify isopod species that may be used as indicators for soil acidity changes.

METHODS

Animals

Isopods were collected from several forests in the Netherlands: Spanderswoud, Roggebotzand and Noord-Hollands Duinreservaat. The pH (H_2O) of the litter at the sites of collection varied from 4.4 to 5.2. Isopods were identified according to Hopkin (1991). Only adult animals were used for the experiments. For *Armadillidium vulgare* experiments were done for males and females separately. For the other species no discrimination to sex was made. Animals were kept in culture pots with litter from the field sites to allow for acclimatisation in a climate room at 19 °C and 70-80 % relative humidity for several days.

Buffer solutions

Buffer solutions were made according to McIlvaine (*table* 1) and were diluted with deionized water to obtain a constant osmolarity for all pH values. The osmolarity was measured using a Wescor osmometer, and was about 170 mOsm L^{-1} for all solutions.

Table 1. – Overview of preparation of McIlvaine buffers, diluted with deionized water to achieve an approximate equal nominal osmolarity of 170 mOsm L^{-1} for all pHs.

nominal pH	Na_2HPO_4 g per 100 ml	$H_3C_6H_5O_7$ g per 100 ml	dilution factor	actual pH in the present experiments
2	0.035	2.431	1	2.20
3	0.733	1.670	1.4	3.11
4	1.376	1.290	2.2	4.10
5	1.840	1.020	2.4	5.13
6	2.254	0.774	2.6	6.13
7	2.937	0.370	2.9	7.06
8	3.470	0.059	3.4	8.33
9	11.000	0.011	11.4	8.96

Preference device

Preference tests where conducted using the devices described in Van Straalen *et al.* (1987). Each unit consists of a perspex circular box of 12 cm diameter, with a bottom and a lid. The bottom is equally divided into 16 compartments (length: 2.4 cm; width: 1.6 cm; depth: 0.8 cm), arranged in a ring, and separated from each other by 2 mm thick dividers. Each compartment may be filled with about 4 g of sand, such that the surface is level with the brim of the dividers. The lid of the box bears two rings which, when the lid is placed on the bottom, form a circular gutter (internal diameter: 7 cm; external diameter: 12 cm) with the ring of 16 compartments in the bottom. The animals may go around freely over the surface of the compartments, without any obstacles.

Dry sand, thoroughly washed with deionized water, obtained from BDH Chemicals, (mesh size 0.2 mm) was added to every compartment up to the brim. After this, buffer solutions were added with 8 different pHs. Each pH was randomly allocated to 2 of the 16 compartments.

Test procedure

To every unit 8 animals of the same species were added. For each species, 12 units were prepared, so the preference was based on 96 individuals per species in total. Individual animals were used only once in the experiments. The preference tests were run in subsequent rounds of 12 units simultaneously, where in each round two or more species were observed. Between rounds, the sand was removed from each compartment and the boxes were thoroughly washed before preparation of a new trial.

When a test was run, the boxes were shielded from sidewards light by placing a circular PVC ring around each (12 cm height), while a mirror mounted above the preference units allowed observations to be made. The tests were done in a climatized room at 19 °C. After 24 h the position of the isopods was noted, disregarding those that were moving around or were on the lid of the box.

Statistical analysis

The observations made on 12 different boxes for each species were pooled into a single frequency distribution. The five distributions obtained were analysed as a contingency table, applying the G-statistic to test the hypothesis of independence of row (species) and column (pH) effects. This was followed by a test on heterogeneity of frequency distributions to establish which species differed from each other (Sokal and Rohlf, 1981).

RESULTS

The frequencies of abundance of individual isopods in the preference units range from pH 2 to pH 9. From *figure* 1 it can be seen that the maximum abundances are at pH 6 for *Porcellio scaber, Oniscus asellus* and *Philoscia muscorum*, and at pH 5 for *Trichoniscus pusillus*. The species *P. scaber* and *O. asellus* are

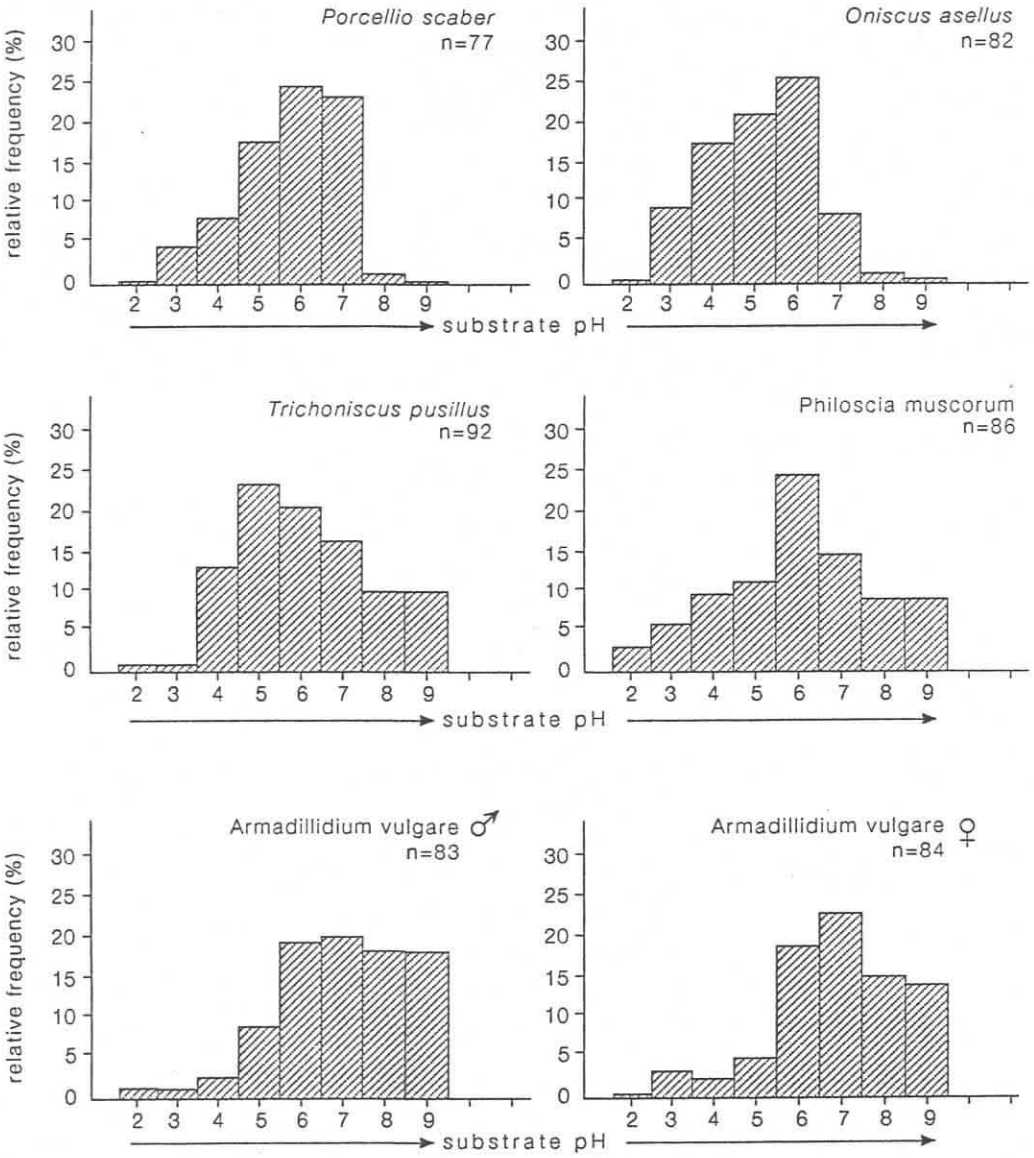

Figure 1. – Pooled relative frequency distributions of five isopod species in pH preference tests run in 12 preference units (each having 8 individuals) for each species. n = number of individuals on which the distribution is based.

observed only rarely at pH 2 and at pH 9, but *T. pusillus* and *P. muscorum* have a more broad distribution. For the species *Armadillidium vulgare* tests were done with males and females separately. From the results no differences between the sexes appeared (*fig.* 1): the maximum abundance is at pH 7 for this species. For all pHs there are isopods present, only at pH 2 *A. vulgare* is rarely seen.

From the G-test it appeared that the frequency distributions of *P. scaber* and *O. asellus* do not differ significantly from each other. Another group is formed by *T. pusillus* and *P. muscorum*, who do not differ significantly from each other but do significantly differ from the first group. *A. vulgare* differs from all others.

To allow the species to be ranked according to their acidophility, we calculated the mean preference for each species as follows:

$$\text{mean preference} = \frac{\Sigma f_i \, \text{pH}_i}{\Sigma f_i}$$

where f_i = the observed frequency at the ith pH, and pH_i = the nominal pH at this frequency. The results are represented in Table 2. It is concluded that the species may be ranked according to their acidophility in the order: *O. asellus, T. pusillus, P. scaber, P. muscorum, A. Vulgare.*

Table 2. – Mean preference for substrate pH for five species of isopods.

Species	Mean preference pH
Oniscus asellus	5.1
Trichoniscus pusillus	5.2
Porcellio scaber	5.7
Philoscia muscorum	5.9
Armadillidium vulgare	7.0

DISCUSSION

From the data and following the G-tests, it can be concluded that the five species of isopods do not react the same to the pH of the substrate. The question may be raised whether the isopods react to pH only or maybe also to other chemical characteristics of the substrate. Other preference experiments (Van Straalen and Verhoef, unpublished) have shown that concentrations of soluble Ca, Al and Fe increase with decreasing pH of the sand-buffer substrate. The observed preference in a pH gradient could actually be due to the isopods selecting an optimal Ca concentration. However, the soluble Ca concentrations at low pH were not particularly high (<200 μM), which makes it unlikely that the avoidance of pH 2 is due to avoidance of Ca. Instead one would expect the isopods to prefer the highest Ca concentration because of their high metabolic needs for this element (Hopkin, 1989). Only a small gradient in Na was present in the buffers due to the dilution to equal osmolarity, therefore Na does not seem to be an important factor in the observed distributions.

The mean preference of the isopods is in the pH range 5-7. This is also observed for several Collembola species investigated with various experimental set-ups (Mertens, 1975; Hutson, 1978; Jaeger and Eisenbeis, 1984; Van Straalen *et al.*, 1987). On the other hand among the oribatid mites there seem to be some species with a pronounced alkalophilic behaviour (Lebrun *et al.*, 1978; Van Straalen *et al.*, 1987).

In the field, isopods have been observed in microhabitats with pHs ranging from 7 to 8 (Ong, 1979). In our case we collected isopods from litter with a much lower pH (5.2 to 4.4). The mean preference observed in the test seems to be one unit higher than the pH of the habitat from wich the animals were collected. However, when the species are put into sequence of decreasing acidophility, the order seems to be grossly in line with field observations. Sutton (1972), in describing the habitat preferences of isopods, mentioned that *P. scaber, O. asellus* and *T. pusillus* are found in a wide range of habitats, even acid moorlands, while *A. vulgare* is frequently seen in stony turf on chalk or limestone.

The biological effects of pH changes in soil cannot be deduced only from behavioural responses. Population changes will occur only if there are negative effects of acidity on survival, growth or reproduction. As *A. vulgare* has the most alkalophilic behaviour, it seems to be the best candidate for further investigating the potential for indicating biological effects of soil acidity changes.

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